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CLONE SPECIFIC SEGREGATION IN THE SEA ANEMONE *ANTHOPLEURA ELEGANTISSIMA*

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The west coast sea anemone *Anthopleura elegantissima* commonly lives intertidally in dense beds. These beds are frequently divided into two or more separate aggregations by anemone-free zones (Fig. 1). These zones may be irregular and rather unremarkable areas occupied by limpets, chitons, snails, barnacles and algae. However, under crowded conditions on inclined surfaces that face toward the sea, these zones are often conspicuous, long, narrow, relatively bare strips anywhere from a fraction of a centimeter to about 5 cm wide (Fig. 1). I have also observed that the position of these zones is relatively constant over periods of up to four years. Whether they are wide or narrow, regular or irregular, these zones separating adjacent aggregations of anemones are apparent to the careful observer because the distance across the zones is obviously greater than the distance between anemones in the midst of the aggregations.

There are also short anemone-free pockets penetrating single aggregations of the anemones. Some of these clearings may be made by the chiton *Mopalia muscosa*, which Field has found to be capable of maintaining and extending artificially created clearings within aggregations of the anemones (Field, Department of Biology, University of California, Santa Barbara, personal communication in 1969), or by the turban snail *Tegula funebralis* which I have observed living in such pockets.

The subject of this paper and associated work (Francis, 1973) is the nature of the relatively permanent anemone-free zones separating adjacent aggregations of the sea anemone *Anthopleura elegantissima* and the relation of these zones to the distribution and behavior of the anemone.

Materials and Methods

Animals and collecting methods

The anemones used in the experiments were all individuals of the aggregating form of *Anthopleura elegantissima* collected intertidally within ten miles of the Hopkins Marine Station at Pacific Grove, California. I removed them from the very large rocks on which they were found by gently working a thin spatula under the edges of the pedal discs.

Laboratory holding conditions

At Hopkins Marine Station the anemones were kept in glass finger-bowls supplied with flowing sea water at about 13° C. The anemones were fed intermittently except during experiments, when food was withheld. Anemones to be used in behavioral experiments were always kept in the laboratory for a few weeks to allow them time to recover from minor damage inflicted during collecting, and to shed the debris that was usually pulled loose from the rocks by the clinging pedal discs.

Only animals that appeared healthy and that attached to the holding bowls by their pedal discs were subsequently used in behavioral experiments. To remove anemones from a holding bowl, I hit the bowl firmly and repeatedly against a hard surface until the anemones released their footholds. Anemones treated in this way appeared to suffer no damage, and they settled in the experimental chambers much more quickly than did anemones that had been forcefully pried loose.

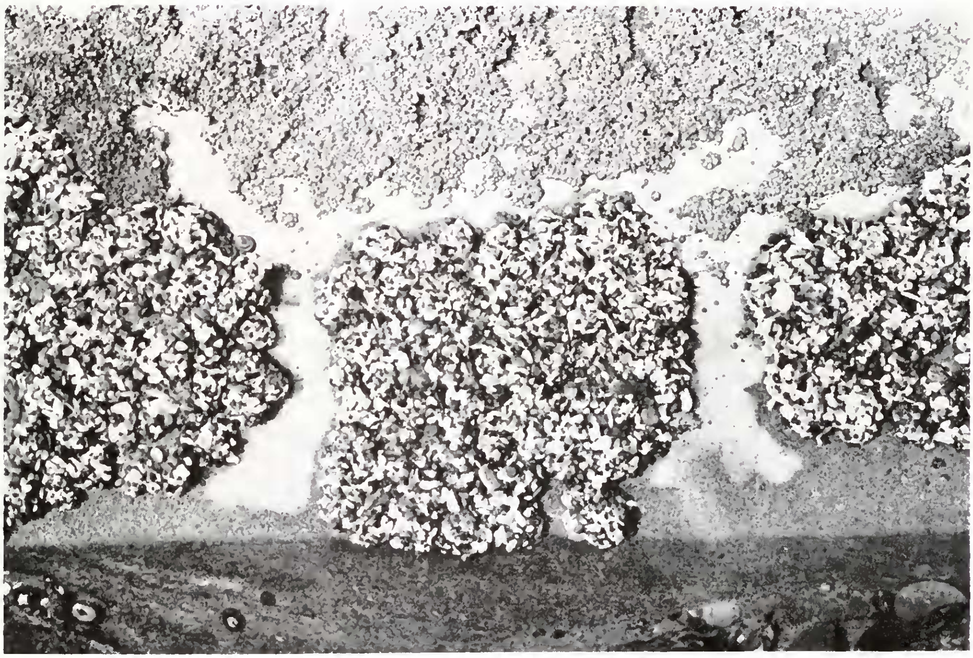

FIGURE 1. A conspicuous anemone-free strip separating adjacent groups (clones) of anemones (intertidal rocks, Arroho Honda, near Baviota, California, December 1970).

Sex determination

The freshly collected anemones were cut in half lengthwise or in the case of larger specimens, in quarters; and the mesenteries were examined for the presence of gonads. During the summer months when the gonads are ripe (Ford, 1964), female gonads are obvious as brownish-pink bodies. Because the yellowish-white male gonads are less easily identifiable, several mesenteries from each non-female anemone were examined microscopically for the presence of spermatocytes. Occasional checks were also made for the presence of oocytes in the smaller female gonads. Anemones having at least one identifiable gonad containing oocytes or spermatocytes were scored as reproductively mature.

Dry weight determination

After sex determination was accomplished, the anemones were blotted using a piece of tissue paper and weighed to ± 0.01 g. The animals were then individually dried to constant weight in a drying oven at between 75° and 91° C (18 to 24 hours were usually required).

PROCEDURES AND RESULTS

Clonal nature of the aggregations

The anemones living in aggregations separated by anemone-free strips were examined closely in order to determine whether those living on opposite sides of the strips differed from each other in any discernible way.

Anthopleura elegantissima shows a variety of color patterning which allows for considerable variation among individuals. For example, a pink pigment is present in the tentacle tips of some but not nearly all individuals. Further, the mesenterial insertion line on the oral disc may be marked with pigment ranging from red-brown to purple in color, and the oral disc may or may not be marked with a radiating white or gold pattern. All of the anemones belonging to a continuous aggregation were observed to have the same color patterning. If more than one color pattern was observed in a bed of anemones on a single rock, the bed was always found to be divided by anemone-free spaces into aggregations of anemones that were segregated by color pattern.

The sexes are separate in this anemone, and samples were taken July 13, 1968 to determine the distribution of the sexes with respect to the anemone-free zones. Samples of at least nine anemones were collected from each of ten contiguous aggregations, each of which was separated from at least one adjacent aggregation by an anemone-free zone. Care was taken in collecting the anemones to select animals from widely separated parts of each aggregation and to include animals from the edges as well as animals from the middle of each aggregation. The results (Table I) show no mixing of the sexes within a single contiguous aggregation. In the single case in which male and female anemones were found living on the same rock, they were found in two unisexual groups separated by an anemone-free strip. In the experiment described below, 99 anemones (a significant fraction of the aggregation) from a single aggregation were collected and examined for the presence of gonads. Of these 21 had female gonads and 78 had no gonads. There were none with male gonads.

Since it is known that these anemones reproduce asexually by longitudinal fission (Hand, 1955), it is apparent that each contiguous aggregation of the sea anemone *Anthopleura elegantissima* is a single clone and that the anemone-free zones described above separate adjacent clones of the anemones. No other hypothesis can simply explain the observed segregation into unisexual groups in which all the group members have the same color patterning.

Size and sexual maturity

It may be noted that some anemones from each clonal group lacked gonads at what is usually the peak period for the gonad index (Ford, 1964).

TABLE I

Distribution of males and females on rocks bearing adjacent aggregations of anemones separated by anemone-free zones

Identifying no. for each aggregation	No. of developed males	No. with sex undeveloped	No. of developed females	Comparison of sex between adjacent aggregations
100	—	8	2	♀/♀
101	—	8	2	
102	—	6	4	♀/♀
103	—	9	1	
104	—	6	3	♀/♀
105	—	8	2	♀/♀
106	—	2	8	
107	7	2	—	♀/♂
108	10	8	—	♂/♂
109	8	2	—	

Both Ford's work and my own casual observation suggested a correlation with the size of the animals. To test this idea, I collected all the anemones (99 anemones) from one section of a female clone on July 29, 1968, and after assaying the reproductive state, I determined the dry weights for each anemone. The results (Table II) show that within this clone, animals smaller than 0.6 g dry weight were not reproductive. The pooled data from smaller samples from 12 different clones, both male and female, collected between July 13 and August 18, 1968, show that none of the individuals below 0.2 g dry weight were reproductive (Table II).

From this it would appear that reporductive state is related to size in these anemones, even among genetically identical individuals, the smallest individuals

TABLE II

Gonad maturity and dry body weight

Whole body dry weight (g)	99 anemones from a single female clone		94 anemones from 12 separate clones both male and female	
	No. of individuals with developed gonads	No. of individuals without developed gonads	No. of individuals with developed gonads	No. of individuals without developed gonads
0.0–0.2	0	16	0	10
0.2–0.4	0	18	4	19
0.4–0.6	0	20	3	10
0.6–0.8	3	10	12	7
0.8–1.0	2	7	4	0
1.0–1.2	8	1	8	2
1.2–1.4	2	2	5	0
1.4–1.6	1	2	5	0
1.6–1.8	1	1	0	0
1.8+	4	1	5	0

being non-reproductive while some proportion of those over 0.2 g dry weight are reproductively mature.

Clonal segregation and separation in the laboratory

Reaggregation into uniclonal groups. An experiment was conducted in order to determine whether or not the observed segregation into clonal groups is a result of an active process carried on by the anemones.

Anemones from two clones (Clone 1 and Clone 2) living adjacent to each other in the field were collected and brought into the laboratory where they were kept with their clonemates in bowls supplied with running sea water.

Ten small to medium sized anemones (from about 0.5 to about 2.3 cm across the expanded oral disc) from each of the two clones were pinned with insect pins to a foam plastic ball about 5 cm in diameter. They were crowded together as closely as possible in five rows of four animals, the individuals from the two clones being arranged alternately in both the horizontal and vertical rows to cover more than half the surface of the ball. This arrangement maximized contact between anemones and allowed contact between both clonemates and non-clonemates. The buoyant ball was fastened to a lead weight using a piece of monofilament line and placed in an aquarium where the ball was held below the surface of the water by the weight. Running sea water at 13° C was continuously supplied to the aquarium. In three days when all the anemones seemed to be adhering to the ball, the pins were removed to allow the anemones to move freely.

After 13 days of free movement, four anemones had fallen off the ball and the remaining 16 anemones had rearranged themselves into two segregated clonal groups. There was at that time no contact between the tentacles of non-clonemates. The anemones continued to move about after 13 days and some further reassortment occurred, but it remained apparent that contact was maintained only between clonemates.

It is apparent from this that clonal segregation within aggregations can be actively accomplished by the anemones.

Formation of an anemone-free trail in the laboratory. The following experiment was designed to determine whether or not the anemones will form anemone-free zones between adjacent clonal groups in the absence of naturally associated species and various environmental factors such as tidal cycle.

The anemones used in the experiment were collected from two different but not adjacent clones (Clone 3 and Clone 4). These animals were maintained in the laboratory for periods varying from several months to a year prior to this experiment. A pyrex baking dish approximately 36 cm by 18 cm and 5 cm deep was lined with a sheet of foam plastic glued to the bottom. One end of the sheet was entirely covered by Clone 3 anemones pinned side by side with insect pins, and the other end was covered with Clone 4 anemones. Where the two clones met at an uneven interface, a barrier of microscope slides fastened together with tape was set on edge between the two clones. The uneveness of the interface served to maximize the area of contact between the clones. Running sea water was introduced into the dish at one end; and because the anemones tend to move upstream (Buchsbaum, 1968), the position of the water inlet was changed periodically to prevent a consistent unidirectional migration in response to current. After a few days when the pedal discs of the anemones had become attached to the substratum, the pins attaching the anemones to the foam plastic sheet were removed as was the barrier between the clones. The animals that released their footholds on the substratum during the rest of the experiment were removed from the dish on the assumption that such animals would have been removed by wave action under field conditions.

Photographs were taken from a fixed position above the dish once a day. The position of the anemones at the beginning of the experiment just after the barrier

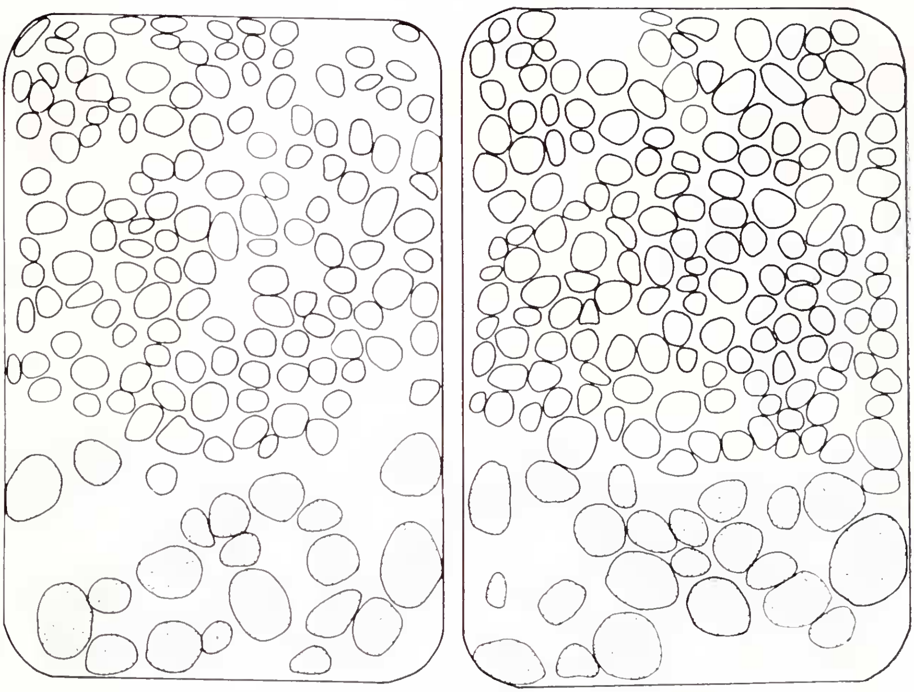

FIGURE 2. The position of anemones from Clone 3 and Clone 4 just after the barrier between the two groups was removed (right) and 14 days after the barrier was removed (left), (see text for details).

was removed and their position 16 days later are shown in Figure 2. The outlines of the oral discs exclusive of tentacles are shown here, and the outlines of the anemones from Clone 3 are shown stippled.

Under these conditions an anemone-free zone was formed between the two clonal groups within three weeks (Fig. 2, left). During this time numerous aggressive episodes (Francis, 1973) were observed at the border between the two groups. (This was not observed during the previous experiment, which was conducted before I discovered the aggressive behavior of this animal.) Apparently then, clonal isolation as well as clonal segregation be actively accomplished by the anemones.

Discussion

The anemones in a single continuous aggregation of the sea anemone *Anthopleura elegantissima* are observed to resemble each other in two obvious ways: (1) all have the same color pattern, and (2) all individuals having developed gonads are of the same sex. Furthermore, while sustained contact between individuals within an aggregation is very common, contact between individuals from different aggregations has been found to initiate an intraspecific aggressive response resulting in the separation of these individuals (Francis, 1973). These animals are known to reproduce asexually by longitudinal fission, and is therefore concluded that the aggregations must each be comprised of anemones from a single clone. It seems to me improbable that anything other than genetic identity could account for aggregations of up to thousands of individuals of the same sex, having the same color patterns and showing mutual tolerance for contact with each other and for no other members of the species.

What might be the advantage to the anemones of living in segregated aggregations? There are a number of possible advantages to a small anemone in living adjacent to other members of the species rather than living alone. (1) Contact between anemones decreases their effective surface areas. This reduces water loss during low tides (Roberts, 1941) and minimizes the area exposed to the pulling and battering effects of the waves and the abrasive effects of suspended matter. (2) An area of rock closely covered by a sheet of anemones provides no place for the settlement of other sessile organisms (such as the larger algae) which may compete with the anemones for space. (3) A single small anemone would have little chance of catching and holding large organisms against the force of the waves; however, I have often seen several anemones in an aggregation together holding and ingesting a large jellyfish or squid.

Anemones of the solitary form of this species are usually larger than individuals of the clonal form. These animals live in more protected circumstances than individuals of the clonal form, usually in nooks and crevices, and often with their bases attached to rocks below the sand level (Hand, 1955). That these larger animals should be found in a more protected habitat than their smaller conspecifics fits well with the preceding analysis. Increased size would make these anemones more vulnerable to the pulling and battering of the waves and therefore less able to occupy flat exposed rock surfaces. At the same time, decrease in the surface to volume ratio of the solitary as compared with the clonal form decreases water loss by evaporation, thus reducing this problem of the solitary living habit. I have observed (as the name of the form itself suggests) that individuals of the solitary form of *Anthopleura elegantissima* also remain isolated from adjacent members of the species.

The advantage to the anemones of segregation into strictly uniclonal groups and separation from adjacent clonal groups is not immediately apparent (see Francis, 1973). However, segregation among genetically different animals of the same species is not peculiar to the species or to the phylum. The growth patterns reported for some colonial coelenterates, tunicates and bryozoans seem to be of the same type.

Schijfsma (1939) reports that young colonies of the hydroid *Hydractinia echinata* seem to fuse completely but that, "When they meet at an older (perhaps different) age a remarkable zone of demarcation is formed," (p. 102). He also notes that when the borders of the same colony meet, for example in growing around a shell, no such line of demarcation is formed.

Theodor (1966) reports a similar phenomenon for the gorgonian *Eunicella stricta.* Two specimens were found growing very close together; and although the bases of the animals were in close contact, the tissues remained unfused. In the laboratory he found that homografts (grafts between genetically different individuals of the same species) always failed to show tissue fusion. Autografts (grafts involving tissue from only one individual) were always successful; and the fused tissues in the graft area appeared normal histologically.

Workers have also observed complete fusion at the interface between separate growing edges of the same colony both in the encrusting ascidian *Botrylus* and in a variety of bryozoa. Knight-Jones and Moyse (1961), referring to these observations, report that, "if two clonies of the same species (of encrusting ascidian or bryozoan) meet, each seems to respect the other's well-marked frontier and spreads only in other directions," (page 88).

What ties these phenomena together with the clone specific segregation and separation reported here for *Anthopleura elegantissima* is the contrast between the intimacy of association among genetically identical "individuals" in colonies or clonal groups, and the relative isolation between genetically different individuals of the same species. All of these animals apparently respond differently to contact with a genetically identical conspecific than to contact with other conspecifics.

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Summary

The anemones in a single continuous aggregation of the sea anemone *Anthopleura elegantissima* are observed to resemble each other in two obvious ways: (1) all have the same color pattern, and (2) all individuals having developed gonads are of the same sex. Furthermore, while sustained contact between individuals within an aggregation is very common, contact between individuals from different aggregations has been found to initiate an intraspecific aggressive response resulting in the separation of these individuals (Francis, 1973). These animals are known to reproduce asexually by longitudinal fission, and it is therefore concluded that the aggregations must each be comprised of anemones from a single clone. It seems to me improbable that anything other than genetic identity could account for aggregations of up to thousands of individuals of the same sex, having the same color patterns and showing mutual tolerance for contact with each other and for no other members of the species.

Even among anemones within a single clone, the presence of gonads containing gametes is shown to be related to size. Anemones smaller than 0.2 g dry wt consistently lack developed gonads, while some proportion of those over 0.2 g have gonads with gametes.

In the field, adjacent clonal groups are observed to remain separated from each other. In the laboratory a mixed group of anemones will reaggregate into isolated uniclonal groups, implying that clone specific segregation is actively accomplished by the anemones.

Living in aggregations has a number of potential advantages for the anemones such as reducing their effective surface area and thereby reducing water loss and the battering effects of wave action, excluding interspecific competition for space by promoting effective blanketing of an area, and allowing for cooperation in the capture and holding of larger prey. The function for the anemones of clone specific segregation and separation is not yet clear (see Francis, 1973), however the phenomenon is not without parallel since some other coelenterates as well as some bryozoans have also been observed to respond differently to contact with a genetically identical conspecific than to contact with other conspecifics.

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